



EXHIBIT A

22 A method of detecting the presence of a carbohydrate antigen characteristic of at least one species or [serogruop] serogroup of a species of bacteria in a fluid, which method comprises the following steps:

[(a) culturing an identified species, or serogroup of a species of bacteria, to a desired size and harvesting therefrom cells of that species, or serogroup of a species of bacteria, as a wet cell pellet;]

[(b)] (a) obtaining from [the wet cell pellet] a culture of a known species, or serogroup of a species of bacteria an essentially protein-free carbohydrate antigen [by a series of substeps which comprise

- (i). suspending the wet cell pellet from step (a) in an alkaline solution and mixing;
- (ii). adjusting the pH to an acid pH with a strong acid;
- (iii). separating the mixture from substep (b) (ii) into two layers;
- (iv). removing the upper layer and adjusting its pH to approximate neutrality;
- (v). adding to the product from substep (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;
- (vi). adjusting the pH of the product from substep (b) (v) to an alkaline pH with a weakly alkaline aqueous solution and;
- (vii). separating out an essentially protein free carbohydrate antigen of said]

species or serogroup of a species of bacteria;]

[(c)] (b) coupling to a chromatographic affinity gel through a spacer molecule the essentially protein-free carbohydrate antigen obtained in step (a) [(b)];

[(d)](c) passing polyclonal antibodies to the same species, or serogroup of a species, of the bacteria, [as that cultured in] referred to in step (a), or an Ig G cut of said antibodies, [thereof] over the chromatographic affinity gel [of] from step (c) to produce purified antigen-specific antibodies; and

[(e)] (d) conducting an assay upon a liquid sample suspected of containing the same species, or serogroup of a species of bacteria, as that [cultured] referred to in step (a), which assay comprises the step of detecting the crude carbohydrate antigen of said species or serogroup of a species of bacteria which is counterpart to the purified antigen of step (a) [(b)], by [contact of] contacting the liquid sample with a detection agent which essentially comprises labelled purified antigen-specific antibodies from step [(d)] (c) hereof, wherein the label may be any known detectable label, and detecting the presence of the suspected, antigen, if present, by detecting a characteristic of the label known to be manifested upon reaction of the labeled antibodies with the suspected antigen.

23 The method of claim 22 in which the species, or serogroup of a species of bacteria, in step (a) are Gram negative bacteria and the crude antigen component thereof sought to be detected in step (d) [(e)] is a lipopolysaccharide.

24 The method of claim 22 in which the species or serogroup of a species of bacteria are Gram positive bacteria and the crude antigen component thereof sought to be detected in step (d) [(e)] is a lipoteichoic acid, a teichoic acid, or a derivative of either.

25 The method of claim 22 in the which the species or serogroup of a species of

bacteria are either Gram negative or Gram positive bacteria and the crude antigen component thereof sought to be detected in step (d) is a capsular polycarbohydrate antigen.

26 The method of claim 22 in which the spacer molecule of step (b) [(c)} is a protein molecule.

27 The method of claim 22 wherein the liquid sample of step (d)[(e)] is water.

28 The method of claim 22 wherein the liquid sample of step (d)[(e)] is a natural fluid of mammalian origin.

29 The method of claim 28 wherein the liquid sample of step (d)[(e)] is human urine.

30 The method of claim 28 wherein the liquid sample of step (d)[(e)] is obtained from a patient exhibiting clinical signs of a disease known to be caused by the bacteria referred to [cultured] in step (a).

31 The method of claim 22 in which step (d)[(e)] is an immunoassay process.

32 The method of claim 31 in which step (d)[(e)] is an immunochromatographic ("ICT") immunoassay process.

33 The method of claim 32 in which the bacteria referred to [cultured] in step (a) are *Haemophilus influenzae* type b bacteria and the crude antigen sought to be detected in step (d) [(e)] is the capsular carbohydrate antigen of those bacteria.

34 The method of claim 22 in which step (d) [(e)] is conducted by

(A) contacting a liquid sample suspected of containing the species, or serogroup of a species, of bacteria referred to [cultured] in step (a) of claim 22, or a crude carbohydrate antigen thereof, with an ICT device comprising a strip of bibulous material, which strip has

(i) a first zone in which has been deposited a movable conjugate of a labelling agent and purified antigen-specific antibodies obtained in step [(d)]

(c) of claim 22, said labelling agent being selected from among those known to display a visible color change upon the formation of a labeled antibody-antigen-fixed antibody reaction product and

(ii) a second zone having immovably bound thereto unconjugated purified antigen-specific antibodies obtained in step [(d)] (c) of claim 22, which zone is equipped with a window for viewing color changes,

(B) allowing said liquid [sample] to flow laterally along said test strip to said first zone, where it picks up the movably deposited conjugate of label and purified antigen-specific antibodies;

(C) allowing said liquid sample and said conjugate of antigen-specific antibodies and label to flow together laterally along said test strip to said second zone, and

(D) within approximately 15 minutes after contacting the liquid sample with the test strip, observing through the aforementioned window whether a line of color indicating the presence in the sample of the suspected bacteria species, or serogroup of a [that] species, has formed.

36 The method of claim 34 in which the bacteria are Gram negative bacteria and the crude antigen sought to be detected is a lipoteichoic acid, a teichoic acid or [an ester] a derivative of either.

43 An ICT device for the detection of a carbohydrate antigen characteristic of a species or serogroup of a species of bacteria, which comprises a strip of bibulous material having

(a) a first zone in which has been movably deposited a conjugate of a labelling agent and purified antibodies specific to the crude carbohydrate antigen of the bacteria

species, or serogroup of a species, suspected of being present in the liquid sample, and

(b) a second zone having immovably bound thereto a portion of unconjugated, purified antibodies specific to the same crude carbohydrate antigen, which zone is equipped with a window for viewing color changes; which device is further characterized in that antigen-specificity of the antibodies present in both zones has been attained by passing polyclonal antibodies to the bacteria species, or serogroup of a species, of which the crude carbohydrate antigen is characteristic over a chromatographic affinity column to which is coupled a spacer molecule conjugated to an essentially protein-free carbohydrate antigen, which essentially protein-free carbohydrate antigen was obtained from a culture of the [*Legionella*] bacteria species, or serogroup of a species of bacteria of which the crude carbohydrate antigen is characteristic, [according to the following method:

- (i) harvesting cells from the culture in the form of a wet cell pellet;
- (ii) suspending the wet cell pellet in an alkaline solution and mixing;
- (iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;
- (iv) separating the acidified product from step (iii) into two layers;
- (v) removing the upper layer and adjusting its pH to approximate neutrality;
- (vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;
- (vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and
- (viii) separating out an essentially protein-free carbohydrate antigen.]

45 The ICT device of claim 43 wherein the species or serogroup of a species of bacteria are Gram negative bacteria and the crude antigen to be detected is a lipoteichoic acid, a teichoic acid or [an ester] a derivative of either.

48 A method for detecting a crude carbohydrate antigen characteristic of a bacteria species, or serogroup of a species, in a liquid sample which comprises the steps of

- (a) contacting said liquid sample with the strip of bibulous material of the ICT device of claim 43;
- (b) allowing said liquid sample to flow laterally along said test strip to the first zone of said device where it picks up a movable deposit of a conjugate of labelling agent and purified antigen-specific antibodies;
- (c) allowing said liquid sample and said conjugate to flow together laterally along said test strip to the second zone of said device; and
- (d) within approximately 15 minutes after contacting the liquid sample with the test strip, observing through the view window whether a line of color has appeared, indicating the presence in the test sample of the species, or serogroup of a species of bacteria, containing the crude carbohydrate antigen to which the purified antibodies are specific.

50 The method of claim 49 wherein the liquid sample is obtained from a human patient exhibiting clinical symptoms of a disease known to be caused by the bacteria species or serogroup of a species of which the crude antigen to be detected is characteristic.